

UV-Killing - Liquid suspensions.

76

Jan. 9, 1947.

Inoculate 5 ml standard suspension in water of Y10.

Plate out .05 ml samples.

T.(secs)	S.		Inoculate flasks at aperture of lamp with halogen by sand.
10	3	{	
20	46	{	
30	1		
45	0		
60	0		
90	0		
120	0		
180	0.		

1/9/47 PM. Repeat.

~~10 sec~~

20 s.

~~30 sec~~

Jan 8, 1947.

as 75. Isolate Y10.

Maltose: 36 plates \times 200 = 7,200 cols.

1. ○ + and - Restreak to purify $\frac{-10^2}{+10^3}$
2. ○ faint +. All -. Pick as W95.
3. ● + and - $\frac{+W97}{-W96}$.
4. ① + and - $\frac{-W98}{+W99}$.
5. ● + and - Restreak to purify $\frac{10^4}{10^5 \pm 10^6} \frac{-}{+}$
6. ● + and - $\frac{u}{-} \frac{10^7}{+}$
7. ● + and - $\frac{-W100}{+W101}$

Wae: 36 plates \times = 7,200 cols.

1. ● + and - $W108-$ $W109^+ +$
2. ○ Resuscitate
3. ○ + and \pm (●). $W110 \pm W111 +$ See 197.
4. ● All +:
5. ● + and - $W112-$ $W113 +$

[Cross-test these].

Jan 9, 1948.

Lactose analogues 1% EMB

		b-Me-galact	b-N-butyl gala.	O-Cresyl-b-galac
Y10	Lac+	++	++	± ^{slow} papillate similar to
Y53	Lac,-	± - + ^{slow}	++	± ^{B-phenyl}
Y35	Lac,-	-	-	- strange inhibition
Y45	Lac,-	-	-	-

The β -N-Butyl galactoside gives the most straightforward differentiation so far noted.

Sucrose & Melibiose & Raffinose.

	Ref 3%	Melibiose st. fil.	Sucrose
"Raf+"	±	slow ++	-
"Raf-"	±	slow +	-
Y40	±	slow +	-

Melibiose activity should be enhanced before attempting to test on raffinose!
Fructose strike filtered.

Y40	+++
W-1	+++

January 4, 1948.

Inoculate YP broths with following:

Y53 (Lac₁-) and:

Cross each on three plates.
A8. (protoplasts +)

nogrowth.

1	W-30		
2	W-35	++	1/3.
3	W-40	+	Like 65. ca 1:100.
4	W-42	-	All - [1/17200 + undet]!
5	W-43	1/100 +	Like 65. ca or < 1:100.
6	W-44		
7	W-45		++ 1/2 - 1/3. See 41
8	W-47		
9	W-48	1/100 +	
10	W-65	-	All - [1 + colony!] 1:100.

Harvest and mix cells. Plate dilute on EMB-Lac(B₁).

∴ None seem to be allelic with Y53. Lac₁-.

a) W35, W45 1/2 - 1/3 Lac+ recombinants

b) W40, W42, W43, W48, W65. ca 1% Lac+ Recombinants.

c) Y53. (Y87?). Original data on Y87 were more limited than these.

Streak out all Lac- and Mal- mutants for recheck!

January 8, 1948

Prepare inocula overnight in YP broth.

Y40 10 AM add 2-3 ml to YP-maltose (A,B) and YP-glucose (C,D) broths.

Incubate W-1 similarly in YP for five hours to 2 PM. Cultures of Y-40 are actively producing gas at this time. Was and cross samples of A,B,C,D, with W-1. Plate on synthetic EM-Maltose(B₁). Count sectors as +.

A: (M2)	M+	M-	% +	S.	
	4	130		0	
	3	78		0	
	3	88		0	
	6	113		1	
	3	156		0	
	9	248		0	
	3	177		0	
	12	398		1	
	7	64		1	
	<hr/>			2.	
					3.099%

B:	O	68	0		
	1	179	0		
	12	435	2		
	7	236	2		
	9	384	0		
	12	284	2		
	1	70	1		
	10	237	2		
	4	135			
	46	2028	2174		2.218%

91	3480	3571	2.548%
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Conclusion: No effect of preadaptation.

- R - S - A - L

Males	8	5	5	0
	7	2	7	2
	15	7	12	2

Wife	8	3	5	0
	4	3	11	1
	8	2	9	1
	8	5	6	0
	6	3	9	0
	9	5	2	0
	11	2	6	0

54. 23 48 2

71 30 60 4
1

60.1 X 10¹²

km/h

C: (G1)	H-	M+	S
1.	8	207	
2.	2	47	2
3.	2	109	2
4.	3	135	
5.	8	267	
6.	2	85	1
7.	2	98	1
8.	0	71	0
9.	22	1019.	41
			total: 1041

D: (G2).	16	269	3
	8	213	3
	3	108	1
	14	357	3
	5	165	
			1153.

41 1112 1153.

63 2131

2194. ~~2.727% Mal+~~
2.871% Mal+.

Comparison:

Glucose.	58.6 ^{59.}	2135	2194.	3806
	63	2131		

91	3480	3571
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154	5611	5765
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$$\chi^2 = 16 \left(\frac{1}{63} + \frac{1}{91} + \frac{1}{2131} + \dots \right)$$

$$\approx .5$$

Mean: Mal+ = $\frac{154}{5765} = .027\%$

Jan 12, 1948

Irradiate .1 ml per plate (LacEMB) 9 secs. under Hanovia.

71 plates x ca. 30 colonies or 2000 colonies.

3 suspicious colonies streaked out:

1:

No mutants

2:

3:

Jan. 13, 1948.

Plate mixtures on Lactose-EMS_{B1}:

	Y87(Lac ₁ -) (>1000) ✓✓	W-45(Lac ₂ -) +++	see 81 ✓ are replicates Lac ₃ - .
Y53			
W108	++✓✓✓	++✓✓✓	
W-112	>1000 ✓✓✓ a	+++ ✓✓✓ b	

On Maltose EMS_{B1}:

	a W56(Mal ₁ -)	b W-60(Mal ₂ -)	
W-1	>1000 ✓	+ ✓	Mal ₁ - : W-1, W-56.
W95	? ±	++	Mal ₂ - : W-60
W-96	±, +	++ ✓	Mal _x - all others.
W98	± (1:1000)	++	
W100	±, -	+ ✓	
W102	±	++	
W104	±	++	
W106	±	++ many scattered.	

+ = 1:100

++ = 1:10

+++ = majority or dominance resp.

Parents checked. p = pigmentation in heavy streaks.

Y53	- p
W45	- p.
W108	- p
Y87	- p. no or few p.
W112	- p. few p.
W102	- p.
W56	- No p.
W98	- p.
W96	- p.
W95	do.
W102	± p

W78. Slow but ++ utilization

W60 = No p.

W20 slow ++ utilization

106 slow + p.
104 slow + p. ++

W71 ± p.

Jan 10 ff 1948

Test strains indicated on T(m) plus .05% substrate.

A. Inulin W-55 Apf (39)

P12 (48h) - -

B. "Bacterial Dextran"
Lot L-10 from
K.P.Link - - A 25 -

Inoc. P12

C. "Soluble Starch"
as above,

A14 ± ++ → dark red-violet.

A17 ± ++

425

W55 fm+ seems to accumulate a red-staining "extinction" from Amylopectin and soluble starch, but utilizes amylose completely.
"Sacharifying amylose ???"

Cross available B-M-Lac - mutants with TLB, Lac, and Lac_3
 tester, ~~W¹¹²~~ ~~T53~~ and W-108

A (1)
 W-112

B (3).
 W-108.

Y87.①

W31	n.c. N.C. [±] . 'col.'	no.col. ✓
W35	+ ✓ ++	
W40.	+ ✓ ++	
W42	✓✓	
W43.	++	
W45①	++ ✓	
W-48	++ ✓	
W55.	+ ✓ also intermediates ??	
W67	-? + ¹⁺ and intermediates?	n.c. ✓ + sm. cols (poor plate).
W72.	n.c. + should be rechecked.	n.c. ✓ n.c.
W74	+* ✓ -	++ ✓ -
W76	+ ✓ ✓	++ ✓ -
W83	+ ✓ +	n.c. ++
W87	+ ✓? + ✓	++ ✓

Jan. 16, 1948.

(Y10)
Suspend cells from plants. Spread on lac EMB (ca. 100,000,000 / pl) and irradiate ~~15 sec.~~ 15 sec. under Stauff's lamps. as above.
x . = colonies.

Run n.g. Evidently, wrong cells (mixture Lac + / Lac -) were used for irradiation.

Jan. 17, 1948.

Glow 12 l. W94 in N2ase 1%, Glucose 1/2% (ster. sep.) and $K_2HPO_4 + KH_2PO_4$ (3:1) .4%. 1 5 gallon Pyrex carboy 24 h. at 37° with aeration.

Collect 53 g. paste in Shaples. Resuspend in .9% NaCl 2 liters and recover 39 g. washed paste.

Mix paste with 2 parts agar⁺ and crush in portions in a Pyrex cone mill, \pm assistance R.H. Burris. Resuspend in 200 cc citrate saline (.1m each). Sediment glass & debris and collect supernatent juice. Add 2 vols alcohol and store in refrigerator. To 100 cc portion. (A)

To remainder, (40 ml.) add $1/3$ v. chloroform + $4\frac{1}{2}$ v. 10 vol. $HgOH$ Mix and store.

(B) A Decant and reject supernatant from A. Sediment and redissolve in 50 ml .1M NaCl. Add 2 vols 95% alcohol in a sterile flask. Repeat. \rightarrow 3.9 gms. alc.-med. paste.

(B). Reject gelled $CHCl_3$ - $HgOH$ -protein. Sediment and decant supernatant. Retreat with $CHCl_3$ overnight. Repeat twice.

Store bulk of extract A. in 95% alcohol.

Suspend 1 gm. paste A in 20 ml NaCl. Add 5 ml aliquots to sterile test tubes and add 10 ml alcohol to each. (use acetone for B²⁴). Allow to stand for sterilization, sediment and replace alcohol with sterile saline, ^{10 ml}. These will contain 1 gm paste / 40 ml saline.

Sol. "A" 90A

B. Third "degassing" → almost clear, opalescent. e.g. liquid. Remove from residual CHCl_3 and ppt. with alcohol 2:1 as above. Sediment and wash with 95% ale. to remove exc. CHCl_3 . Suspend sediment in 10 ml H₂O, add 5 x alcohol. Ppt. fibrous. lift out with glass rod and resuspend in 1M NaCl → clear but str. opalescent solution.

Repeat with remainder of sediment. Gave very little ^{aliquot} fibrous sediment, considerable granular which is thrown out. Final suspension presumably polymeric NH. in 10 ml NaCl. "~~Sol. B~~". Sediment with 5 vols. alcohol in sterile tubes, and resuspend in sterile NaCl, 40 ml.. "Sol B." 90B.

Note N²⁴, 1 tube of B pstd with 2 vols. alcohol. No fibrous ppt. formed suggesting depolymerization.

TP Activity.

January 19, 1948.

Add 1 ml. 90A + B. resp to 10 ml YB broth tubes (5 ea.).

Use 2 for sterility tests. Inoculate each of the other three with 98 hr. culture Y138. Also 3 tubes of C suis for no-treatment controls.

Read A 20.

1	A1		all Mal +
2	A2		all Mal +. (A Phage plaque?)
3	A3		all Mal +
4	A8t	turbid	Some very fine Not coli
5	A8t	turbid.	No colonies Some very fine. Not coli
6	B1		All Mal +.
7	B2		"
8	B3		"
9	B8t	Turbid!	Cult. Not coli..
10	B8t.	Clear	No colonies
11	C1		All Mal +
12	C2		"
13	C3.		"

Streak out all tubes on Mal ~~and~~ EMBS.

→ ~~Streak out~~ Test on ~~EMBS~~ Y138

	O	A	L
1-1	0, C, 0	0	11, 15
1-2			
1-3			
2-1	0, C, 0	0, 0	5, 1
3-1	0, 0, 0	0, 0	15, 15
4-1	heavily loaded with actinomycete contaminant		
5-1	heavily contaminated.		
6-1	0	0	34
6-2	0	0	3
6-3	0	0	0
7-1	0	3	30
8-1	0	0	45
9-1	{ loaded with "inoculated" contaminant.		
9-2			
9-3			
11-1	0	0, 1	16, 1
11-2	0	0	1
11-3	0	0	0
12-1	0	1	32
13-1	0	0	46, 26

There is no evidence from this experiment of transformation of the A - or L - loci either by the crude extracts or by the fibrous material of 'B'.

Replate cells in series 1 in A + L agar.

Find and plot the growth curves of the cells of late.

Preparation of T.P.

94

Jan 23, 1948.

Grow W-94 "anaerobically" in 12 l. N₂ case medium, 24 h.
37°. Yield: 17 g. Sharples paste (~~1/3~~ 1/3 aerobic yield).
Suspend in 170 ml NaCl (physiological) & blend c. 2 ml volume.
Let stand 4 hours, sediment + ppt. supernatant c. 2 1/2 vols 95% alc.
V. little sediment formed. Separate + store in 70% alcohol. (C)

Jan 27, 1948

Streak out the following "inversions" of W108 on the ^{homologous} medium, as indicated, to purify.

From Glucose. EMBS plates of 93. - to lactose + maltose.

	L	M	
+	Y10	Y10	
3			Test 31 "inversions" on glucose plates on
4			lactose and on maltose.
5			All 31 glucose-inversions are also lactose + maltose +.
6			
7			
8			
9			

plates M1, M2, L1, L2

From Lac + Mal EMBS. Streak out to Lac + Mal + Mann.

+ Man.

10 Mal + are Lac +
6 Lac + are Mal +.

L, M, Mann = ¹Man + also Mal + and Lac +.
3/4 Man + are weaks", fourth is strong.
Purify + compare \approx Y10.

From 93 Broth 108M / M + L resp.

From 93 T(m). Maltose 108M (Tm) / M + L resp.

\rightarrow 10 + L +

All inversions are non-specific for glucose, maltose + lactose

No. tested:

Glucose 31

Lactose 6

Select. are as W-108^R = W116.

Maltose 13

Mannitol 4

54 tested altogether.

Characterization of W-108.

96

January 28, 1948.

T(m) + : .05% ^{T₁₀₈} w₁₀₈ (autobalanced together). Y10.

glucose.	-	+++
D-hexose	-	+
hexose diphosphate.	++	++.
" + glucose		+++.

The HDP was prepared from the Schenay 2a salt product by adding excess oxalate and neutralizing with NaOH. The solution contains exc. oxalate, which is evidently not inhibitory considering the control. In autoclaving, the HDP solution turns quite yellow so that breakdown must be suspected. Repeat expts. using filter sterilized HDP.

Test Proteins X-19 on HDP. Add to T(m)+ mi:

	A29.	A2
glucose	-	++
fructose	-	-
HDP.	-	++

Jan 29, 1948.

S. dublin I IX g,p; - Arab - B, -
X

S. paratyphi A. I II XII a; - Ar+ B,+ Meth-Trypt -.

on arabinose minimal medium.

Mix sep. + together into YP broth. ① S1 ② S37 ③ S1+S37^X

(A) Plate .1ml washed samples of 16 hr. cultures in arabinose T(m) minimal.
 1. S1 12 cols. 4. S1+S37. 2. S37 S1 revert on ~~the~~ arabinose minimal.
 3. X ca 10-20 cols.

(B). Do.

1. S1 0 2 large many small,
1 mm.

2. S37 0,0

3. X 0, 3 mm. cols., 10 cols., 0, 0

4. S1 + S37 3 c., 10-20 c., 100 c., 100 c. many small.
100%.

Read 2/4/48.

(4) may represent a revo. Add'l differentiating
character used to eliminate S1 revert.

Jan. 29, 1948.

Test 93. W108: glu+ and tre+ on glucose & fructose EMB.

1. Glut. On 2 TMB, all visible colonies with on glucose + fructose.
2. Tre+ in T(m). Both grow rapidly on glucose, fairly rapidly on ~~glucose~~ fructose, T+ both.

Switch from Glucose plates to EMB glucose.

- (1) —
(2) — in 24 hours.

Take 99-1, impure, as W-117

W-117 is either an aerobic oxidizer of glucose or else a slow fermenter.

Compare on glucose and on K-gluconate:

W117:	EMB:	
	glucose	+ weak +. Use these colonies for pure W-117
	Maltose	± - +
	Lactose	-
	K-glucon.	+++